Supplementary – Methods

Immunohistochemical (IHC) analyses

Routine hematoxilin-eosin was performed to evaluate tissue structures. IHC studies were performed in a subset of 14 formalin-fixed paraffin-embedded available tumor samples, after technique standardization in testicular tissues (Supplementary Figure 1).

Briefly, slides were deparaffinized and tissue sections were rehydrated, antigen retrieval in citrate buffer solution (pH 6.0) for 40 minutes, incubated in 3% H₂O₂ for 30 minutes, normal horse serum (1:50) for 1 hour and BSA 1% for 15 minutes. Slides were then incubated in primary antibodies anti-SMO (sc-13943; 1:50), anti-GLI1 (sc-20687; 1:30), anti-GLI3 (sc-20688; 1:100), anti-SUFU (sc-28847; 1:500), anti-beta-catenin (sc-7963; 1:200; Santa Cruz Biotechnology Inc.) and Ki67 (NCL-Ki67-MM1; 1:200; Novocastra™, UK) at 4°C, overnight, incubated in secondary antibody for 30 minutes, followed by signal detection by avidin-biotin system (Vectastain Elite ABC Kit; Vector Laboratories Inc., Burlingame, CA) according to the manufacturer’s instructions. The staining was developed with DAB (3,3’-diaminobenzidine-tetrahydrochloride; Vector Laboratories Inc.) and counterstained with Harris hematoxylin. As negative controls, all specimens were incubated with an isotope-matched control antibody under identical conditions. Preparations for each marker were evaluated randomly in at least 10 representative high-power fields (X400). The immunolabeling percentage was evaluated by a ratio of unequivocal nuclei labeling in 100 counted cells or percentage of labeled area using the Image J software (National Institutes of Health, Bethesda, MD).

FIGURE LEGEND

Supplementary Figure 1: Illustrative images of immunohistochemical technique standardization of SMO, GLI1, GLI3 and SUFU proteins in testicular tissue (Scale 100µm).